Multicenter study to evaluate diagnostic methods for detection and isolation of *Campylobacter* from stool

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Modified Abstract

Background: The use of culture independent methods as standalone tests for the direct detection of *Campylobacter* in stool is increasing. We conducted a prospective, multicenter study to evaluate the real-world performance of stool antigen assays in comparison to culture and PCR for detection of *Campylobacter* from stool.

Methods: Stool specimens collected between July and October 2010 from patients in 8 states who were being evaluated for GI illness were tested with the following methods: four *Campylobacter* selective media (CVA, Campy-cefex, mCCDA and CSM), four EIA assays (ProSpecT ™ Campylobacter, PREMIER™ CAMPY, ImmunoCard STAT! (ICS) and Xpect Campy) and PCR (Seegene). Clinical and epidemiologic data were collected from patients whose stool was positive in any of the tests performed.

Results: A total of 3.2 % (88/2767) of specimens tested were positive by culture, of which 13.6% (12/88) were negative in all 4 EIA tests. As compared to culture, the sensitivity/specificity/positive predictive values of the EIA tests were: Premier CAMPY, 82.6/97.3/51.7%, ProSpecT, 83.7 /97.7/55.7%, ICS, 73.1/96.1/39.8% and XpecT Campy, 74.2/99.4/80.2%. Of the 206 culture-negative specimens that were positive in one or more of the EIA tests, only 2.9% (6/206) were positive in all four EIA tests, and 76.2% (157/206) were positive in only one of the four EIA tests: ICS (n=67), ProSpecT (n=44), Premier CAMPY (n=36), XpecT Campy (n=10). All but one of these 157 specimens was PCR negative. We found significant differences in demographics and clinical symptoms between cases and non- cases.

Conclusions: While convenient to use, the sensitivity and specificity of *Campylobacter* EIA tests was variable. Given the low incidence of *Campylobacter* disease and the performance data generated in this study, we recommend EIA tests should not be used as standalone tests for direct detection of *Campylobacter* in stool.

Laboratory diagnosis of *Campylobacter* infection

The problems

- There are currently no national clinical or state public health best practice guidelines for *Campylobacter* diagnostic testing
- The use of stool antigen tests is increasing
  - Guidelines for interpretation and reporting discordant results between non-culture test and culture results for a given specimen do not exist.
  - Current *Campylobacter* case definition requires culture confirmation
- There are no evaluations of the current gold standard – culture

Aim of Study

- We conducted a prospective, multicenter study to evaluate the real-world performance of stool antigen assays in comparison to culture and PCR for detection of *Campylobacter* from stool.

Results

- **A total of 3.2% (88/2767) of specimens were positive by culture**

Number of culture positive specimens(n=88)	Premier CAMPY	ProSpecT	ICS	XpecT Campy	PCR
61	**P**	**P**	**P**	**P**	**P**(n=57), Neg (n=3), NT (n=1)
12	Neg	Neg	Neg	Neg	**P**(n=5), Neg (n=6), NT (n=1)
1	**P**	Neg	Neg	Neg	**P**
2	Neg	**P**	Neg	Neg	Neg
5	**P**	**P**	Neg	Neg	**P** (n=4), Neg (n=1)
1	Neg	**P**	Neg	**P**	**P**
2	**P**	**P**	Neg	**P**	**P**
2	**P**	**P**	**P**	Neg	**P**
1	**P**	Neg	**P**	**P**	**P**
1	NT	NT	Neg	Neg	Neg
Nos. of false negatives	15	14	24	23	13

- **225 culture negative specimens were positive in at least one test**

# culture negative	Premier CAMPY	ProSpecT	ICS	XpecT Campy	PCR
4	**P**	**P**	**P**	**P**	**P**
1	Neg	P	Neg	Neg	**P**
2	**P**	**P**	**P**	**P**	Neg
67	Neg	Neg/ Ind	**P**	Neg/ Inv	Neg
44	Neg	**P**	Neg	Neg/ inv	Neg
36	**P**	Neg	Neg	Neg	Neg
10	Neg	Neg/ Ind	Neg	**P**	Neg
5	**P**	**P**	Neg	Neg	Neg
6	Neg	**P**	**P**	Neg/ Inv	Neg
3	Neg	**P**	Neg	**P**	Neg
24	**P**	Neg/ Ind	**P**	Neg	Neg
2	**P**	**P**	**P**	Neg	Neg
2	**P**	Neg/ Ind	**P**	**P**	Neg
19	Neg	Neg	Neg	Neg	**P**
False positives?	71	62	103	17	19

- **Performance characteristics of culture and stool antigen tests based on study case definition**

	Sensitivity	Specificity	PPV	NPV
Premier CAMPY	**82.6%**	**97.3%**	**51.7%**	**99.4%**
ProSpecT	**83.7%**	**97.7%**	**55.7%**	**99.5%**
ICS	**73.1%**	**96.1%**	**39.8%**	**99.0%**
XpecT Campy	**74.2%**	**99.4%**	**80.2%**	**99.1%**
PCR	**85.7%**	**99.3%**	**80.4%**	**99.5%**
Culture	**94.6%**	**100%**	**100%**	**99.8%**

- **Comparison of Epidemiologic Characteristics of *Campylobacter* Cases and Non-Cases**
  - Epidemiologic data is currently available for 212/313 patients that were positive in at least one of the tests performed

	Cases (n=91)			Non-Cases (n=142)			p-value
	#	Total	(%)	#	Total	(%)	
Age: median (range)	26 yrs (25 days-77yrs)			44.5 yrs (26 days-100yrs)			**0.0341**
Sex: Female	36	87	(41.4%)	76	134	(56.7%)	**0.0259**
Race: White	60	74	(81.1%)	80	102	(78.4%)	0.6671
Ethnicity: Hispanic	10	73	(13.7%)	11	102	(10.8%)	0.5586
Diarrhea	67	69	(97.1%)	77	92	(83.7%)	**0.0062**
Bloody diarrhea	24	60	(40.0%)	34	74	(45.9%)	0.4897
Fever	44	64	(68.8%)	39	86	(45.3%)	**0.0044**
Nausea	36	62	(58.1%)	45	84	(53.6%)	0.5892
Vomiting	25	66	(37.9%)	31	90	(34.4%)	0.6586
Abdominal cramps	54	62	(87.1%)	62	83	(74.7%)	0.0648
Days of illness: med (range)	10 (5-29)			20 (3-60)			**0.0179**
Hospitalized	27	78	(34.6%)	56	102	(54.9%)	**0.0068**
Prior antibiotic	4	62	(6.5%)	21	85	(24.7%)	**0.0036**

- Non-cases were significantly more likely than cases to be older, female, hospitalized, haven taken antibiotics prior to illness and have a longer duration of illness; and less likely to have had diarrhea or fever.

Study Design

- **Study Sites:** Stool specimens were collected from participating study site hospital, county or state public health laboratories in eight states (CA, CO, CT, GA, IA, MD, MN and PA) from patients with GI illness, on whom routine enteric diagnostic laboratory testing, including *Campylobacter* testing had been ordered.
- **Study Period**: July to October 2010
- **Methods tested:**
  - Four *Campylobacter* selective media (CVA, Campy-cefex, mCCDA and CSM)
  - Four EIA assays: ProSpecT ™ Campylobacter (Remel), PREMIER™ CAMPY(Meridian Bioscience), ImmunoCard STAT! (ICS, Meridian Bioscience) and Xpect Campy (Remel)
  - PCR (Seegene Inc - Multiplex assay that also detects *Salmonella, Shigella, Vibrio* and *Clostridium difficile* toxin B)

  All commercial EIA and PCR methods were performed according to manufacturer's instructions
- **Associated Data:** Clinical and epidemiologic data were collected from patients whose stool was positive in any of the tests performed.
- **Case Definition :** Specimen is culture positive OR Specimen is positive in at least one stool antigen test and the PCR

Conclusions / Next steps

- While convenient to use, the performance characteristics of *Campylobacter* EIA tests was variable.
- Given the low incidence of *Campylobacter* disease and the performance data generated in this study, we recommend that EIA tests should not be used as standalone tests for direct detection of *Campylobacter* in stool.
  - a positive EIA test alone is not sufficient to consider a case "confirmed"
  - laboratories should confirm positive EIA results by culture
- Next steps include:
  i) Establishing *Campylobacter* Clinical and Public Health workgroups to develop best practice guidelines for *Campylobacter* diagnostic testing.
  ii) Review of current *Campylobacter* case definition; update if appropriate.

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